

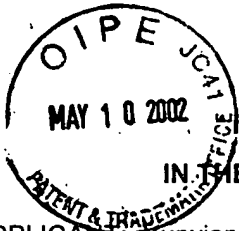
references to newly added Figures 1a, 1b and 1c have been added to the specification at pages 10 and 14. No new matter has been added.

Should the Examiner have any questions, he or she is urged to call Applicants' undersigned attorney, Tara A. Nealey, at 314-872-8118.

Respectfully submitted,



Tara A. Nealey  
Registration No. 42,927  
POLSTER, LIEDER, WOODRUFF  
& LUCCHESI, L.C.  
763 South New Ballas Road  
St. Louis, MO 63141  
(314) 872-8118  
(314) 991-2178 (fax)



ZHUY 08216  
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Yunxiang Zhu.

ART UNIT: 1621

SERIAL NO.: 10/051,711

EXAMINER:

FILED: January 17, 2002

DOCKET NO.: ZHUY 8216US

FOR: (AS AMENDED) METHODS FOR INTRODUCING MANNOSE 6-PHOSPHATE AND  
OTHER OLIGOSACCHARIDES ONTO GLYCOPROTEINS

RECEIVED  
MAY 15 2002  
TECH CENTER 1600-2900

Assistant Commissioner for Patents  
Box Non-fee Amendment  
Washington, D.C. 20231

**APPENDIX TO AMENDMENT**

**Version of Paragraphs and Sections in Specification with Markings to Show  
Changes Made**

Commissioner for Patents  
Box Fee Amendment  
Washington, D.C. 20231

**IN THE TITLE:**

Page 1, line 8 is amended as follows: METHODS FOR INTRODUCING  
MANNOSE 6-PHOSPHATE AND OTHER OLIGOSACCHARIDES ONTO  
GLYCOPROTEINS [AND ITS APPLICATION THEREOF]

**IN THE DRAWINGS:**

New drawing sheet 2/6 with Figures 1a, 1b and 1c are added.

**IN THE SPECIFICATION:**

Page 2, please delete lines 1 and 2.

Page 10, please delete the section beginning line 19 and insert:

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a schematic representation of the conjugation methods;

Figures 1a, 1b, and 1c are schematic representations of alternative oxidation methods for the conjugation methods;

Figure 2 is a SDS-PAGE analysis of different avidins before and after phosphopentamannose-hydrazine conjugation;

Figure 3 is a CI-MPR binding analysis of untreated avidin and oxidized avidin conjugated with phosphopentamannose-hydrazine;

Figure 4 is a bar graph showing enzymatic activity of beta-glucuronidase after different treatments including after conjugation; and

Figure 5 is a bar graph comparing CI-MPR binding of untreated and oxidized phosphopentamannose-conjugated beta-glucuronidase.

Page14, please delete the paragraph beginning line 14, and insert:

Figure 1 is a schematic representation of the conjugation methods. In a first step, the reducing terminal sugar of oligosaccharides is derivatized to glycosylhydrazine (as shown) or other carbonyl-reactive groups (such as hydrazide, semicarbozide, aminooxyl, etc.). Such oligosaccharides must have one or more phosphate groups attached to the C 6' position(s) on mannopyranosyl groups (M6P). The oligosaccharide derivatives then react with the carbonyl (aldehyde) groups generated in the oxidized carbohydrates on glycoproteins to form covalent bond conjugates. The glycoproteins are oxidized according to one of at least three possible methods, as shown in Figures 1a, 1b and 1c. By a first method, sialic acids on glycans are oxidized with a low

concentration of sodium periodate (less than or equal to 10mM) to generate the required carbonyl groups. A second method is suitable when terminal galactoses exist on the glycans, in which enzymatic oxidation is used. More specifically, galactose oxidase is used to oxidize the C 6' hydroxyl group on the galactose groups. This second oxidation method should not inactivate the glycoprotein. In an alternative embodiment of the second oxidation method, sialic acid groups on glycoprotein carbohydrates are removed using neuraminidase to expose the terminal galactoses, and then galactose oxidase is used to oxidize the terminal exposed galactoses as described for the first embodiment of the second oxidation method. By a third oxidation method, the hexoses on the glycans are oxidized with relatively high concentrations of sodium periodate, i.e. with sodium periodate having a concentration of greater than about 10 mM and less than about 500 mM, to open the vicinal hydroxyl groups of the sugar ring. This third oxidation method is potentially harmful to certain glycoproteins that are sensitive to oxidation. To protect the glycoproteins from oxidation of amino acids, reductive agents such as beta-mecaptoenthanol or cysteine or others are added to the oxidation reaction.